



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of Ian Farquhar Campbell
and Mauro Sergio Sandrin

Art Unit 1632

Serial No.: 09/051,034:

Filed: 31 March 1998

For: Improved Nucleic Acids encoding a
Chimeric glycosyltransferase

DECLARATION

Hon. Commissioner of Patents and Trademarks
Washington, DC 2021

SIR:

I, Mauro Sergio Sandrin of 211 Barkly Street, Brunswick 3056, Victoria,
Australia do solemnly and sincerely declare as follows:

1. I am currently employed by The Austin Research Institute as Professor and Senior Research Principal Fellow
2. My educational background is as follows: I have a Ph.D. in immunogenetics, a D.Sc in molecular biology. I have research experience in these areas, and in the areas of glycobiology and xenotransplantation.
3. I am the second named inventor of the above captioned US Patent Application (hereinafter referred to as "the present application") which is presently under final rejection.
4. I have reviewed the final Office Action dated 30 January 2001.
5. I believe that it is not correct that the present invention extends only to specific chimeric glycosyltransferases such as gHTT and pgHTT (see Official Action page 8 last paragraph to page 12, 2nd paragraph).
6. In the following paragraphs I set out details of experiments carried out under my supervision. I believe that these experiments demonstrate that chimeric glycosyltransferases other than those specifically exemplified in the present application are able to reduce the gal epitope in accordance with the invention.

RECEIVED
APR 04 2001
TECH CENTER 1632

7. (i) The attached Figures show that other chimeric glycosyltransferases constructed in accordance with the invention claimed have the ability to reduce the gal epitope.
- (ii) Figure 1 shows COS cells transfected with various glycosyltransferases attached to Flagtags that enable the cells to be analysed by immunofluorescence with anti-Flag mAb/sheep anti-mouse FITC:

Panel A shows the localisation of the tail and transmembrane regions of pig fucosyltransferase 1 (ft or ht) in the Golgi.

Panel B shows fluorescent staining of cells transfected with Secretor (secretory fucosyltransferase or Se) - the Se is confined to the cytoplasm of the Cos cells and

Panel C shows the staining pattern of cells transfected with a chimera consisting of an ft tail followed by the transmembrane and catalytic domains of Se (ftSe). The presence of the ft tail localised the Se to the Golgi.

8. (i) Figure 1 shows that the use of a glycosyltransferase other than gt is capable of locating a different enzyme to the Golgi. The figure shows that ftSe is located in the Golgi. Se is a pig fucosyltransferase that can use an acceptor substrate in common with $\alpha(1,3)$ galactosyltransferase. Cells transfected with both H (a fucosyltransferase 1) and $\alpha(1,3)$ galactosyltransferase have reduced amounts of the gal epitope because the H competes with the galactosyltransferase for a common acceptor or substrate (see for example, the paragraph bridging pages 2 and 3 of the present application). The location of Se in the Golgi by the ft tail (or cytoplasmic domain) would thus enable it to compete with the $\alpha(1,3)$ galactosyltransferase, thereby decreasing the gal epitope in accordance with the present invention.
- (ii) Figure 2 also shows the ability of other chimeric transferases to decrease the gal epitope. Chinese hamster ovary cells (CHOPs) were transfected with 1) gt and Se or 2) gt and ft(tail)-Se (that is, gt and a chimeric molecule of ft- tail together with Se catalytic domain), as shown in panels B and C respectively. Control cells were transfected with full length gt and pcDNA (pc DNA being a vector) or pcDNA alone (panels A and D respectively).
- (iii) Cells were surface stained with IB4-FITC to detect the Gal α 1,3Gal epitope. The population of cells which are positive are shown in marker set 2 and 3 (M2 and M3 respectively). In controls cells that were gt transfected with vector (pcDNA) there were 343 mfu (mean fluorescence units) in marker set 3 and when gt was transfected with Se this was reduced to 241 mfu. When the ft tail was attached to the Se transmembrane and catalytic domain, IB4 staining was markedly reduced and the staining was 170 mfu, showing the reduction in gal epitope production. Furthermore, the number of positively stained

cells in marker set 3 reduced from 34% for Se to only 3% for the chimera, ft(tail) Se.

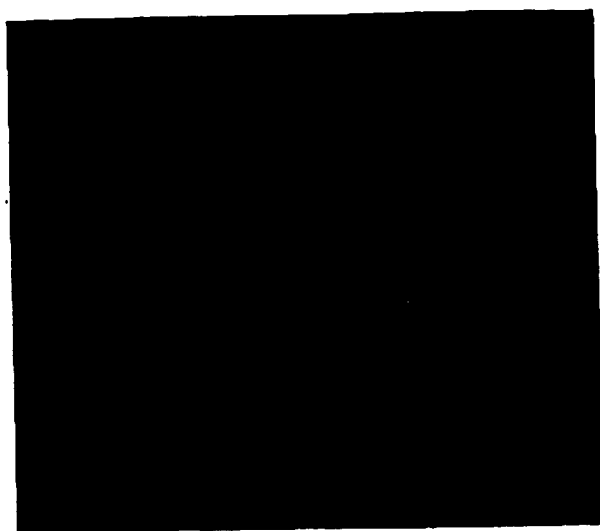
- (iv) These results suggests that Se and the ft (tail)-Se chimera both compete for acceptor molecules with $\alpha(1,3)$ galactosyltransferase to form the Gal $\alpha(1,3)$ Gal epitope, with ft-Se reducing the gal epitope to a much greater extent. This is due to the altered localisation of the Se and the ft (tail)-Se to the Golgi where the enzyme is in a better position to contact, and compete for, acceptor substrate in accordance with the invention claimed in the present application.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States code, and that such wilful statements may jeopardise the validity of the application or any patent issuing thereon.

DATED this 29 day of MARCH, 2001



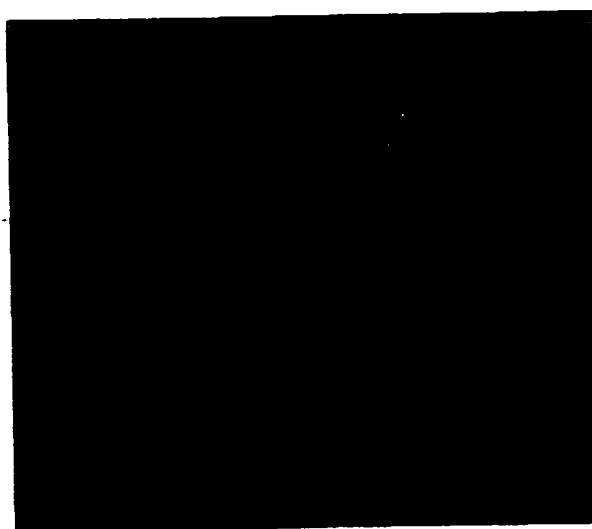
MAURO SERGIO SANDRIN



A



B

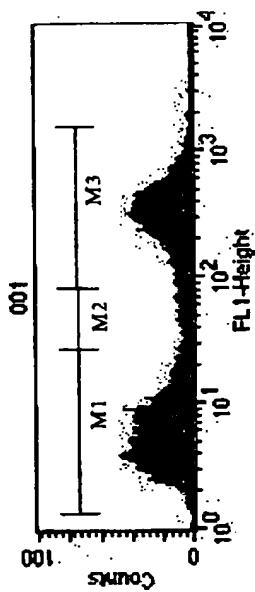


C

FIGURE 1: COS-7 cells were transfected with DNA encoding the FT tail and transmembrane followed by a Flag-tag (panel A), full length secretor followed by a Flag-tag (panel B) or FT tail followed by the transmembrane and catalytic domain of secretor (panel C). The cells were permeabilised and stained with anti-Flag mAb (Sigma) followed by sheep anti-mouse-FITC (Silenus).

The FT tail and transmembrane were localised in the Golgi as previously shown (panel A). Secretor was difficult to detect or gave a cytoplasmic staining pattern (panel B), however when the FT tail was attached to secretor it localised to the Golgi (panel C), confirming that the FT tail is sufficient for Golgi localisation.

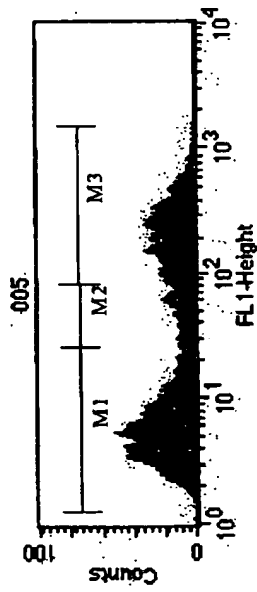
Figure 2
A: GT+pcDNA
IB4-FITC



File: 001 Acquisition Date: 14-Sep-0
Gate: G1

Marker	% Gated	Mean	Geo Mean	Median
All	100.00	144.91	31.19	12.98
M1	55.23	6.87	5.76	5.47
M2	4.54	50.29	47.62	49.14
M3	40.19	343.03	302.58	313.40

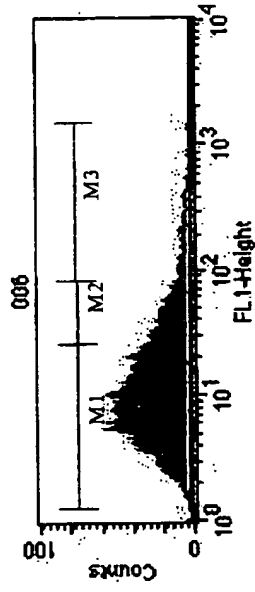
B: GT+Se
IB4-FITC



File: 005 Acquisition Date: 14-Sep-0
Gate: G1

Marker	% Gated	Mean	Geo Mean	Median
All	100.00	92.90	25.04	13.34
M1	55.54	6.99	5.87	5.52
M2	9.90	52.79	50.13	53.28
M3	34.67	241.91	210.79	210.97

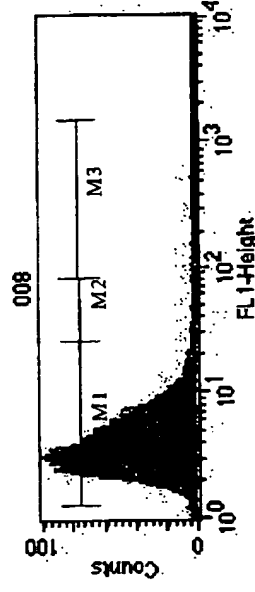
C: GT+FTailSe
IB4-FITC



File: 006 Acquisition Date: 14-Sep-0
Gate: G1

Marker	% Gated	Mean	Geo Mean	Median
All	100.00	21.87	11.96	10.46
M1	79.68	9.91	8.22	8.28
M2	16.57	42.87	40.79	39.24
M3	3.93	179.75	153.60	137.00

D: pcDNA
IB4-FITC



File: 008 Acquisition Date: 14-Sep-0
Gate: G1

Marker	% Gated	Mean	Geo Mean	Median
All	100.00	4.68	4.02	3.72
M1	99.61	4.51	4.00	3.68
M2	0.18	34.86	33.41	31.06
M3	0.07	188.74	153.79	119.71